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(54) **Title:** METHODS FOR GENERATING GENETICALLY ALTERED ANTIBODY-PRODUCING CELL LINES WITH IMPROVED ANTIBODY CHARACTERISTICS

(57) **Abstract:** Dominant negative alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepared more efficiently than by relying on the natural rate of mutation. These methods are useful for generating genetic diversity within immunoglobulin genes directed against an antigen of interest to produce altered antibodies with enhanced biochemical activity. Moreover, these methods are useful for generating antibody-producing cells with increased level of antibody production.

METHODS FOR GENERATING GENETICALLY ALTERED ANTIBODY-PRODUCING CELL LINES WITH IMPROVED ANTIBODY CHARACTERISTICS

TECHNICAL FIELD OF THE INVENTION

The invention is related to the area of antibody maturation and cellular production. In particular, it is related to the field of mutagenesis.

BACKGROUND OF THE INVENTION

The use of antibodies to block the activity of foreign and/or endogenous polypeptides provides an effective and selective strategy for treating the underlying cause of disease. In particular is the use of monoclonal antibodies (MAb) as effective therapeutics such as the FDA approved ReoPro (Glaser, V. (1996) Can ReoPro repolish tarnished monoclonal therapeutics? *Nat. Biotechnol.* 14:1216-1217), an anti-platelet MAb from Centocor; Herceptin (Weiner, L.M. (1999) Monoclonal antibody therapy of cancer. *Semin. Oncol.* 26:43-51), an anti-Her2/neu MAb from Genentech; and Synagis (Saez-Llorens, X.E., *et al.* (1998) Safety and pharmacokinetics of an intramuscular humanized monoclonal antibody to respiratory syncytial virus in premature infants and infants with bronchopulmonary dysplasia. *Pediat. Infect. Dis. J.* 17:787-791), an anti-respiratory syncytial virus MAb produced by Medimmune.

Standard methods for generating MAbs against candidate protein targets are known by those skilled in the art. Briefly, rodents such as mice or rats are injected with a purified antigen in the presence of adjuvant to generate an immune response (Shield, C.F., *et al.* (1996) A cost-effective analysis of OKT3 induction therapy in cadaveric kidney transplantation. *Am. J. Kidney Dis.* 27:855-864). Rodents with positive immune sera are sacrificed and splenocytes are isolated. Isolated splenocytes are fused to melanomas to produce immortalized cell lines that are then screened for antibody production. Positive lines are isolated and characterized for antibody production. The direct use of rodent MAbs as human therapeutic agents were confounded by the fact that human anti-rodent antibody (HARA) responses occurred in a significant number of patients treated with the rodent-derived antibody (Khazaeli, M.B., *et al.*, (1994) Human immune response to monoclonal antibodies. *J. Immunother.* 15:42-52). In order to circumvent the problem of HARA, the grafting of the complementarity determining regions (CDRs), which are the

critical motifs found within the heavy and light chain variable regions of the immunoglobulin (Ig) subunits making up the antigen binding domain, onto a human antibody backbone found these chimeric molecules are able to retain their binding activity to antigen while lacking the HARA response (Emery, S.C., and Harris, W.J. "Strategies for humanizing antibodies" In: ANTIBODY ENGINEERING C.A.K. Borrebaeck (Ed.) Oxford University Press, N.Y. 1995. pp. 159-183. A common problem that exists during the "humanization" of rodent-derived MAbs (referred to hereon as HAb) is the loss of binding affinity due to conformational changes in the 3 dimensional structure of the CDR domain upon grafting onto the human Ig backbone (U.S. Patent No. 5,530,101 to Queen *et al.*). To overcome this problem, additional HAb vectors are usually needed to be engineeredby inserting or deleting additional amino acid residues within the framework region and/or within the CDR coding region itself in order to recreate high affinity HAbs (U.S. Patent No. 5,530,101 to Queen *et al.*). This process is a very time consuming procedure that involves the use of expensive computer modeling programs to predict changes that may lead to a high affinity HAb. In some instances the affinity of the HAb is never restored to that of the MAb, rendering them of little therapeutic use.

Another problem that exists in antibody engineering is the generation of stable, high yielding producer cell lines that is required for manufacturing of the molecule for clinical materials. Several strategies have been adopted in standard practice by those skilled in the art to circumvent this problem. One method is the use of Chinese Hamster Ovary (CHO) cells transfected with exogenous Ig fusion genes containing the grafted human light and heavy chains to produce whole antibodies or single chain antibodies, which are a chimeric molecule containing both light and heavy chains that form an antigen-binding polypeptide (Reff, M.E. (1993) High-level production of recombinant immunoglobulins in mammalian cells. *Curr. Opin. Biotechnol.* 4:573-576). Another method employs the use of human lymphocytes derived from transgenic mice containing a human grafted immune system or transgenic mice containing a human Ig gene repertoire. Yet another method employs the use of monkeys to produce primate MAbs, which have been reported to lack a human anti-monkey response (Neuberger, M., and Gruggermann, M. (1997) Monoclonal antibodies. Mice perform a human repertoire. *Nature* 386:25-26). In all cases, the generation of a cell line that is capable of generating sufficient amounts of high affinity antibody poses a major limitation for producing sufficient materials for clinical studies. Because of these limitations, the utility of other recombinant systems such

as plants are currently being explored as systems that will lead to the stable, high-level production of humanized antibodies (Fiedler, U., and Conrad, U. (1995) High-level production and long-term storage of engineered antibodies in transgenic tobacco seeds. *Bio/Technology* 13:1090-1093).

A method for generating diverse antibody sequences within the variable domain that results in HAbs and MAbs with high binding affinities to antigens would be useful for the creation of more potent therapeutic and diagnostic reagents respectively. Moreover, the generation of randomly altered nucleotide and polypeptide residues throughout an entire antibody molecule will result in new reagents that are less antigenic and/or have beneficial pharmacokinetic properties. The invention described herein is directed to the use of random genetic mutation throughout an antibody structure *in vivo* by blocking the endogenous mismatch repair (MMR) activity of a host cell producing immunoglobulins that encode biochemically active antibodies. The invention also relates to methods for repeated *in vivo* genetic alterations and selection for antibodies with enhanced binding and pharmacokinetic profiles.

In addition, the ability to develop genetically altered host cells that are capable of secreting increased amounts of antibody will also provide a valuable method for creating cell hosts for product development. The invention described herein is directed to the creation of genetically altered cell hosts with increased antibody production via the blockade of MMR.

The invention facilitates the generation of high affinity antibodies and the production of cell lines with elevated levels of antibody production. Other advantages of the present invention are described in the examples and figures described herein.

SUMMARY OF THE INVENTION

The invention provides methods for generating genetically altered antibodies (including single chain molecules) and antibody producing cell hosts *in vitro* and *in vivo*, whereby the antibody possess a desired biochemical property(s), such as, but not limited to, increased antigen binding, increased gene expression, and/or enhanced extracellular secretion by the cell host. One method for identifying antibodies with increased binding activity or cells with increased antibody production is through the screening of MMR defective antibody producing cell clones that produce molecules with enhanced binding properties or clones that have been genetically altered to produce enhanced amounts of antibody product.

The antibody producing cells suitable for use in the invention include, but are not limited to rodent, primate, or human hybridomas or lymphoblastoids; mammalian cells transfected and expressing exogenous Ig subunits or chimeric single chain molecules; plant cells, yeast or bacteria transfected and expressing exogenous Ig subunits or chimeric single chain molecules.

Thus, the invention provides methods for making hypermutable antibody-producing cells by introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into cells that are capable of producing antibodies. The cells that are capable of producing antibodies include cells that naturally produce antibodies, and cells that are engineered to produce antibodies through the introduction of immunoglobulin encoding sequences. Conveniently, the introduction of polynucleotide sequences into cells is accomplished by transfection.

The invention also provides methods of making hypermutable antibody producing cells by introducing a dominant negative mismatch repair (MMR) gene such as *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2* into cells that are capable of producing antibodies. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*). The invention also provides methods in which mismatch repair gene activity is suppressed. This may be accomplished, for example, using antisense molecules directed against the mismatch repair gene or transcripts.

Other embodiments of the invention provide methods for making a hypermutable antibody producing cells by introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into fertilized eggs of animals. These methods may also include subsequently implanting the eggs into pseudo-pregnant females whereby the fertilized eggs develop into a mature transgenic animal. The mismatch repair genes may include, for example, *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2*. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*).

The invention further provides homogeneous compositions of cultured, hypermutable, mammalian cells that are capable of producing antibodies and contain a dominant negative allele of a mismatch repair gene. The mismatch repair genes may

include, for example, *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2*. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*). The cells of the culture may contain *PMS2*, (preferably human *PMS2*), *MLH1*, or *PMS1*; or express a human *mutL* homolog, or the first 133 amino acids of hPMS2.

The invention further provides methods for generating a mutation in an immunoglobulin gene of interest by culturing an immunoglobulin producing cell selected for an immunoglobulin of interest wherein the cell contains a dominant negative allele of a mismatch repair gene. The properties of the immunoglobulin produced from the cells can be assayed to ascertain whether the immunoglobulin gene harbors a mutation. The assay may be directed to analyzing a polynucleotide encoding the immunoglobulin, or may be directed to the immunoglobulin polypeptide itself.

The invention also provides methods for generating a mutation in a gene affecting antibody production in an antibody-producing cell by culturing the cell expressing a dominant negative allele of a mismatch repair gene, and testing the cell to determine whether the cell harbors mutations within the gene of interest, such that a new biochemical feature (*e.g.*, over-expression and/or secretion of immunoglobulin products) is generated. The testing may include analysis of the steady state expression of the immunoglobulin gene of interest, and/or analysis of the amount of secreted protein encoded by the immunoglobulin gene of interest. The invention also embraces prokaryotic and eukaryotic transgenic cells made by this process, including cells from rodents, non-human primates and humans.

Other aspects of the invention encompass methods of reversibly altering the hypermutability of an antibody producing cell, in which an inducible vector containing a dominant negative allele of a mismatch repair gene operably linked to an inducible promoter is introduced into an antibody-producing cell. The cell is treated with an inducing agent to express the dominant negative mismatch repair gene (which can be *PMS2* (preferably human *PMS2*), *MLH1*, or *PMS1*). Alternatively, the cell may be induced to express a human *mutL* homolog or the first 133 amino acids of hPMS2. In another embodiment, the cells may be rendered capable of producing antibodies by co-transfecting a preselected immunoglobulin gene of interest. The immunoglobulin genes of the hypermutable cells, or the proteins produced by these methods may be analyzed for desired

properties, and induction may be stopped such that the genetic stability of the host cell is restored.

The invention also embraces methods of producing genetically altered antibodies by transfecting a polynucleotide encoding an immunoglobulin protein into a cell containing a dominant negative mismatch repair gene (either naturally or in which the dominant negative mismatch repair gene was introduced into the cell), culturing the cell to allow the immunoglobulin gene to become mutated and produce a mutant immunoglobulin, screening for a desirable property of said mutant immunoglobulin protein, isolating the polynucleotide molecule encoding the selected mutant immunoglobulin possessing the desired property, and transfecting said mutant polynucleotide into a genetically stable cell, such that the mutant antibody is consistently produced without further genetic alteration. The dominant negative mismatch repair gene may be *PMS2* (preferably human *PMS2*), *MLH1*, or *PMS1*. Alternatively, the cell may express a human *mutL* homolog or the first 133 amino acids of hPMS2.

The invention further provides methods for generating genetically altered cell lines that express enhanced amounts of an antigen binding polypeptide. These antigen-binding polyeptides may be, for example, immunoglobulins. The methods of the invention also include methods for generating genetically altered cell lines that secrete enhanced amounts of an antigen binding polypeptide. The cell lines are rendered hypermutable by dominant negative mismatch repair genes that provide an enhanced rate of genetic hypermutation in a cell producing antigen-binding polypeptides such as antibodies. Such cells include, but are not limited to hybridomas. Expression of enhanced amounts of antigen binding polypeptides may be through enhanced transcription or translation of the polynucleotides encoding the antigen binding polypeptides, or through the enhanced secretion of the antigen binding polypeptides, for example.

Methods are also provided for creating genetically altered antibodies *in vivo* by blocking the MMR activity of the cell host, or by transfecting genes encoding for immunoglobulin in a MMR defective cell host.

Antibodies with increased binding properties to an antigen due to genetic changes within the variable domain are provided in methods of the invention that block endogenous MMR of the cell host. Antibodies with increased binding properties to an antigen due to genetic changes within the CDR regions within the light and/or heavy

chains are also provided in methods of the invention that block endogenous MMR of the cell host.

The invention provides methods of creating genetically altered antibodies in MMR defective Ab producer cell lines with enhanced pharmacokinetic properties in host organisms including but not limited to rodents, primates, and man.

These and other aspects of the invention are provided by one or more of the embodiments described below. In one embodiment of the invention, a method for making an antibody producing cell line hypermutable is provided. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into an antibody-producing cell. The cell becomes hypermutable as a result of the introduction of the gene.

In another embodiment of the invention, a method is provided for introducing a mutation into an endogenous gene encoding for an immunoglobulin polypeptide or a single chain antibody. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction and expression of the MMR gene allele. The cell further comprises an immunoglobulin gene of interest. The cell is grown and tested to determine whether the gene encoding for an immunoglobulin or a single chain antibody of interest harbors a mutation. In another aspect of the invention, the gene encoding the mutated immunoglobulin polypeptide or single chain antibody may be isolated and expressed in a genetically stable cell. In a preferred embodiment, the mutated antibody is screened for at least one desirable property such as, but not limited to, enhanced binding characteristics.

In another embodiment of the invention, a gene or set of genes encoding for Ig light and heavy chains or a combination therein are introduced into a mammalian cell host that is MMR defective. The cell is grown, and clones are analyzed for antibodies with enhanced binding characteristics.

In another embodiment of the invention, a method will be provided for producing new phenotypes of a cell. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell is grown. The cell is tested for the expression of new phenotypes where the phenotype is enhanced secretion of a polypeptide.

These and other embodiments of the invention provide the art with methods that can generate enhanced mutability in cells and animals as well as providing cells and

animals harboring potentially useful mutations for the large-scale production of high affinity antibodies with beneficial pharmacokinetic profiles.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Hybridoma cells stably expressing PMS2 and PMS134 MMR genes. Shown is steady state mRNA expression of MMR genes transfected into a murine hybridoma cell line. Stable expression was found after 3 months of continuous growth. The (-) lanes represent negative controls where no reverse transcriptase was added, and the (+) lanes represent samples reverse transcribed and PCR amplified for the MMR genes and an internal housekeeping gene as a control.

Figure 2. Creation of genetically hypermutable hybridoma cells. Dominant negative MMR gene alleles were expressed in cells expressing a MMR-sensitive reporter gene. Dominant negative alleles such as PMS134 and the expression of MMR genes from other species results in antibody producer cells with a hypermutable phenotype that can be used to produce genetically altered immunoglobulin genes with enhanced biochemical features as well as lines with increased Ig expression and/or secretion. Values shown represent the amount of converted CPRG substrate which is reflective of the amount of function □-galactosidase contained within the cell from genetic alterations within the pCAR-OF reporter gene. Higher amounts of β-galactosidase activity reflect a higher mutation rate due to defective MMR.

Figure 3. Screening method for identifying antibody-producing cells containing antibodies with increased binding activity and/or increased expression/secretion

Figure 4. Generation of a genetically altered antibody with an increased binding activity. Shown are ELISA values from 96-well plates, screened for antibodies specific to hIgE. Two clones with a high binding value were found in HB134 cultures.

Figure 5. Sequence alteration within variable chain of an antibody (a mutation within the light chain variable region in MMR-defective HB134 antibody producer cells). Arrows indicate the nucleotide at which a mutation occurred in a subset of cells from a clone derived from HB134 cells. Panel A: The change results in a Thr to Ser change within the light chain variable region. The coding sequence is in the antisense direction. Panel B: The change results in a Pro to His change within the light chain variable region.

Figure 6. Generation of MMR-defective clones with enhanced steady state Ig

protein levels. A Western blot of heavy chain immunglobulins from HB134 clones with high levels of MAb (>500ngs/ml) within the conditioned medium shows that a subset of clones express higher steady state levels of immunoglobulins (Ig). The H36 cell line was used as a control to measure steady state levels in the parental strain. Lane 1: fibroblast cells (negative control); Lane 2: H36 cell; Lane 3: HB134 clone with elevated MAb levels; Lane 4: HB134 clone with elevated MAb levels; Lane 5: HB134 clone with elevated MAb levels.

Methods have been discovered for developing hypermutable antibody-producing cells by taking advantage of the conserved mismatch repair (MMR) process of host cells. Dominant negative alleles of such genes, when introduced into cells or transgenic animals, increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable. Hypermutable cells or animals can then be utilized to develop new mutations in a gene of interest. Blocking MMR in antibody-producing cells such as but not limited to: hybridomas; mammalian cells transfected with genes encoding for Ig light and heavy chains; mammalian cells transfected with genes encoding for single chain antibodies; eukaryotic cells transfected with Ig genes, can enhance the rate of mutation within these cells leading to clones that have enhanced antibody production and/or cells containing genetically altered antibodies with enhanced biochemical properties such as increased antigen binding. The process of MMR, also called mismatch proofreading, is carried out by protein complexes in cells ranging from bacteria to mammalian cells. A MMR gene is a gene that encodes for one of the proteins of such a mismatch repair complex. Although not wanting to be bound by any particular theory of mechanism of action, a MMR complex is believed to detect distortions of the DNA helix resulting from non-complementary pairing of nucleotide bases. The non-complementary base on the newer DNA strand is excised, and the excised base is replaced with the appropriate base, which is complementary to the older DNA strand. In this way, cells eliminate many mutations that occur as a result of mistakes in DNA replication.

Dominant negative alleles cause a MMR defective phenotype even in the presence of a wild-type allele in the same cell. An example of a dominant negative allele of a MMR gene is the human gene *hPMS2-134*, which carries a truncating mutation at codon 134 (SEQ ID NO:15). The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations,

which accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity, even in the presence of the wild-type allele. Any allele which produces such effect can be used in this invention. Dominant negative alleles of a MMR gene can be obtained from the cells of humans, animals, yeast, bacteria, or other organisms. Such alleles can be identified by screening cells for defective MMR activity. Cells from animals or humans with cancer can be screened for defective mismatch repair. Cells from colon cancer patients may be particularly useful. Genomic DNA, cDNA, or mRNA from any cell encoding a MMR protein can be analyzed for variations from the wild type sequence. Dominant negative alleles of a MMR gene can also be created artificially, for example, by producing variants of the *hPMS2-134* allele or other MMR genes. Various techniques of site-directed mutagenesis can be used. The suitability of such alleles, whether natural or artificial, for use in generating hypermutable cells or animals can be evaluated by testing the mismatch repair activity caused by the allele in the presence of one or more wild-type alleles, to determine if it is a dominant negative allele.

A cell or an animal into which a dominant negative allele of a mismatch repair gene has been introduced will become hypermutable. This means that the spontaneous mutation rate of such cells or animals is elevated compared to cells or animals without such alleles. The degree of elevation of the spontaneous mutation rate can be at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold that of the normal cell or animal. The use of chemical mutagens such as but limited to methane sulfonate, dimethyl sulfonate, O6-methyl benzadine, MNU, ENU, etc. can be used in MMR defective cells to increase the rates an additional 10 to 100 fold that of the MMR deficiency itself.

According to one aspect of the invention, a polynucleotide encoding for a dominant negative form of a MMR protein is introduced into a cell. The gene can be any dominant negative allele encoding a protein, which is part of a MMR complex, for example, *PMS2, PMS1, MLH1*, or *MSH2*. The dominant negative allele can be naturally occurring or made in the laboratory. The polynucleotide can be in the form of genomic DNA, cDNA, RNA, or a chemically synthesized polynucleotide.

The polynucleotide can be cloned into an expression vector containing a constitutively active promoter segment (such as but not limited to CMV, SV40, Elongation Factor or LTR sequences) or to inducible promoter sequences such as the steroid inducible

pIND vector (Invitrogen), where the expression of the dominant negative MMR gene can be regulated. The polynucleotide can be introduced into the cell by transfection.

According to another aspect of the invention, an immunoglobulin (Ig) gene, a set of Ig genes or a chimeric gene containing whole or parts of an Ig gene can be transfected into MMR deficient cell hosts, the cell is grown and screened for clones containing genetically altered Ig genes with new biochemical features. MMR defective cells may be of human, primates, mammals, rodent, plant, yeast or of the prokaryotic kingdom. The mutated gene encoding the Ig with new biochemical features may be isolated from the respective clones and introduced into genetically stable cells (*i.e.*, cells with normal MMR) to provide clones that consistently produce Ig with the new biochemical features. The method of isolating the Ig gene encoding Ig with new biochemical features may be any method known in the art. Introduction of the isolated polynucleotide encoding the Ig with new biochemical features may also be performed using any method known in the art, including, but not limited to transfection of an expression vector containing the polynucleotide encoding the Ig with new biochemical features. As an alternative to transfecting an Ig gene, a set of Ig genes or a chimeric gene containing whole or parts of an Ig gene into an MMR deficient host cell, such Ig genes may be transfected simultaneously with a gene encoding a dominant negative mismatch repair gene into a genetically stable cell to render the cell hypermutable.

Transfection is any process whereby a polynucleotide is introduced into a cell. The process of transfection can be carried out in a living animal, *e.g.*, using a vector for gene therapy, or it can be carried out *in vitro*, *e.g.*, using a suspension of one or more isolated cells in culture. The cell can be any type of eukaryotic cell, including, for example, cells isolated from humans or other primates, mammals or other vertebrates, invertebrates, and single celled organisms such as protozoa, yeast, or bacteria.

In general, transfection will be carried out using a suspension of cells, or a single cell, but other methods can also be applied as long as a sufficient fraction of the treated cells or tissue incorporates the polynucleotide so as to allow transfected cells to be grown and utilized. The protein product of the polynucleotide may be transiently or stably expressed in the cell. Techniques for transfection are well known. Available techniques for introducing polynucleotides include but are not limited to electroporation, transduction, cell fusion, the use of calcium chloride, and packaging of the polynucleotide together with lipid for fusion with the cells of interest. Once a cell has been transfected with the MMR

gene, the cell can be grown and reproduced in culture. If the transfection is stable, such that the gene is expressed at a consistent level for many cell generations, then a cell line results.

An isolated cell is a cell obtained from a tissue of humans or animals by mechanically separating out individual cells and transferring them to a suitable cell culture medium, either with or without pretreatment of the tissue with enzymes, *e.g.*, collagenase or trypsin. Such isolated cells are typically cultured in the absence of other types of cells. Cells selected for the introduction of a dominant negative allele of a mismatch repair gene may be derived from a eukaryotic organism in the form of a primary cell culture or an immortalized cell line, or may be derived from suspensions of single-celled organisms.

A polynucleotide encoding for a dominant negative form of a MMR protein can be introduced into the genome of an animal by producing a transgenic animal. The animal can be any species for which suitable techniques are available to produce transgenic animals. For example, transgenic animals can be prepared from domestic livestock, *e.g.*, bovine, swine, sheep, goats, horses, etc.; from animals used for the production of recombinant proteins, *e.g.*, bovine, swine, or goats that express a recombinant polypeptide in their milk; or experimental animals for research or product testing, *e.g.*, mice, rats, guinea pigs, hamsters, rabbits, etc. Cell lines that are determined to be MMR defective can then be used as a source for producing genetically altered immunoglobulin genes *in vitro* by introducing whole, intact immunoglobulin genes and/or chimeric genes encoding for single chain antibodies into MMR defective cells from any tissue of the MMR defective animal.

Once a transfected cell line or a colony of transgenic animals has been produced, it can be used to generate new mutations in one or more gene(s) of interest. A gene of interest can be any gene naturally possessed by the cell line or transgenic animal or introduced into the cell line or transgenic animal. An advantage of using such cells or animals to induce mutations is that the cell or animal need not be exposed to mutagenic chemicals or radiation, which may have secondary harmful effects, both on the object of the exposure and on the workers. However, chemical mutagens may be used in combination with MMR deficiency, which renders such mutagens less toxic due to an undetermined mechanism. Hypermutable animals can then be bred and selected for those producing genetically variable B-cells that may be isolated and cloned to identify new cell lines that are useful for producing genetically variable cells. Once a new trait is identified,

the dominant negative MMR gene allele can be removed by directly knocking out the allele by technologies used by those skilled in the art or by breeding to mates lacking the dominant negative allele to select for offspring with a desired trait and a stable genome. Another alternative is to use a CRE-LOX expression system, whereby the dominant negative allele is spliced from the animal genome once an animal containing a genetically diverse immunoglobulin profile has been established. Yet another alternative is the use of inducible vectors such as the steroid induced pIND (Invitrogen) or pMAM (Clonetech) vectors which express exogenous genes in the presence of corticosteroids.

Mutations can be detected by analyzing for alterations in the genotype of the cells or animals, for example by examining the sequence of genomic DNA, cDNA, messenger RNA, or amino acids associated with the gene of interest. Mutations can also be detected by screening for the production of antibody titers. A mutant polypeptide can be detected by identifying alterations in electrophoretic mobility, spectroscopic properties, or other physical or structural characteristics of a protein encoded by a mutant gene. One can also screen for altered function of the protein *in situ*, in isolated form, or in model systems. One can screen for alteration of any property of the cell or animal associated with the function of the gene of interest, such as but not limited to Ig secretion.

Examples of mismatch repair proteins and nucleic acid sequences include the following:

PMS2 (mouse) (SEQ ID NO:5)

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MEQTEGVSTE CAKAIKPIDG KSVHQICSGQ VILSLSTAVK ELIENSVDAG ATTIDLRLKD  60
YGVDLIEVSD NGCGVEEENF EGLALKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
TISTCHGSAS VGTRLVFDHN GKITQKTPYP RPKGTTVSVQ HLFYTLPVRY KEFQRNIKKE 180
YSKMVQVLQA YCIISAGVRV SCTNQLGQGK RHAVVCTSGT SGMKENIGSV FGQKQLQSLI 240
PFVQLPPSDA VCEEYGLSTS GRHKTFSTFR ASFHSARTAP GGVQQTGSFS SSIRGPVTQQ 300
RSLSLSMRFY HMYNRHQYPF VVLNVSVDSE CVDINVTPDK RQILLQEEKL LLAVLKTSLI 360
GMFDSDANKL NVNQQPLLDV EGNLVKLHTA ELEKPVPGKQ DNSPSLKSTA DEKRVASISR 420
LREAFSLHPT KEIKSRGPET AELTRSFPSE KRGVLSSYPS DVISYRGLRG SQDKLVSPTD 480
SPGDCMDREK IEKDSGLSST SAGSEEEFST PEVASSFSSD YNVSSLEDRP SQETINCGDL 540
DCRPPGTGQS LKPEDHGYQC KALPLARLSP TNAKRFKTEE RPSNVNISQR LPGPQSTSAA 600
EVDVAIKMNK RIVLLEFSLS SLAKRMKQLQ HLKAQNKHEL SYRKFRAKIC PGENQAAEDE 660
LRKEISKSMF AEMEILGQFN LGFIVTKLKE DLFLVDQHAA DEKYNFEMLQ QHTVLQAQRL 720
ITPQTLNLTA VNEAVLIENL EIFRKNGFDF VIDEDAPVTE RAKLISLPTS KNWTFGPQDI 780
DELIFMLSDS PGVMCRPSRV RQMFASRACR KSVMIGTALN ASEMKKLITH MGEMDHPWNC 840
PHGRPTMRHV ANLDVISQN                                             859
```

PMS2 (mouse cDNA) (SEQ ID NO:6)

```
gaattccggt gaaggtcctg aagaatttcc agattcctga gtatcattgg aggagacaga 60
taacctgtcg tcaggtaacg atggtgtata tgcaacagaa atgggtgttc ctggagacgc 120
gtcttttccc gagagcggca ccgcaactct cccgcggtga ctgtgactgg aggagtcctg 180
catccatgga gcaaaccgaa ggcgtgagta cagaatgtgc taaggccatc aagcctattg 240
atgggaagtc agtccatcaa atttgttctg ggcaggtgat actcagttta agcaccgctg 300
tgaaggagtt gatagaaaat agtgtagatg ctggtgctac tactattgat ctaaggctta 360
```

```
aagactatgg ggtggacctc attgaagttt cagacaatgg atgtggggta gaagaagaaa 420
actttgaagg tctagctctg aaacatcaca catctaagat tcaagagttt gccgacctca 480
cgcaggttga aactttcggc tttcgggggg aagctctgag ctctctgtgt gcactaagtg 540
atgtcactat atctacctgc cacgggtctg caagcgttgg gactcgactg gtgtttgacc 600
ataatgggaa aatcacccag aaaactccct acccccgacc taaaggaacc acagtcagtg 660
tgcagcactt attttataca ctacccgtgc gttacaaaga gtttcagagg aacattaaaa 720
aggagtattc caaaatggtg caggtcttac aggcgtactg tatcatctca gcaggcgtcc 780
gtgtaagctg cactaatcag ctcggacagg ggaagcggca cgctgtggtg tgcacaagcg 840
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tcattccttt tgttcagctg ccccctagtg acgctgtgtg tgaagagtac ggcctgagca 960
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PMS2 (human) (SEQ ID NO:7)

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MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENSLDAG ATNIDLKLKD 60
YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
TISTCHASAK VGTRLMFDHN GKIIQKTPYP RPRGTTVSVQ QLFSTLPVRH KEFQRNIKKE 180
YAKMVQVLHA YCIISAGIRV SCTNQLGQGK RQPVVCTGGS PSIKENIGSV FGQKQLQSLI 240
PFVQLPPSDS VCEEYGLSCS DALHNLFYIS GFISQCTHGV GRSSTDRQFF FINRRPCDPA 300
KVCRLVNEVY HMYNRHQYPF VVLNISVDSE CVDINVTPDK RQILLQEEKL LLAVLKTSLI 360
GMFDSDVNKL NVSQQPLLDV EGNLIKMHAA DLEKPMVEKQ DQSPSLRTGE EKKDVSISRL 420
REAFSLRHTT ENKPHSPKTP EPRRSPLGQK RGMLSSSTSG AISDKGVLRP QKEAVSSSHG 480
PSDPTDRAEV EKDSGHGSTS VDSEGFSIPD TGSHCSSEYA ASSPGDRGSQ EHVDSQEKAP 540
ETDDSFSDVD CHSNQEDTGC KFRVLPQPTN LATPNTKRFK KEEILSSSDI CQKLVNTQDM 600
SASQVDVAVK INKKVVPLDF SMSSLAKRIK QLHHEAQQSE GEQNYRKFRA KICPGENQAA 660
EDELRKEISK TMFAEMEIIG QFNLGFIITK LNEDIFIVDQ HATDEKYNFE MLQQHTVLQG 720
QRLIAPQTLN LTAVNEAVLI ENLEIFRKNG FDFVIDENAP VTERAKLISL PTSKNWTFGP 780
QDVDELIFML SDSPGVMCRP SRVKQMFASR ACRKSVMIGT ALNTSEMKKL ITHMGEMDHP 840
WNCPHGRPTM RHIANLGVIS QN                                         862
```

PMS2 (human cDNA) (SEQ ID NO:8)

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ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180
aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
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cctgtggtat gcacaggtgg aagccccagc ataaaggaaa atatcggctc tgtgtttggg 720
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cttttcaaac c 2771
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PMS1 (human) (SEQ ID NO:9)

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MKQLPAATVR LLSSSQIITS VVSVVKELIE NSLDAGATSV DVKLENYGFD KIEVRDNGEG 60
IKAVDAPVMA MKYYTSKINS HEDLENLTTY GFRGEALGSI CCIAEVLITT RTAADNFSTQ 120
YVLDGSGHIL SQKPSHLGQG TTVTALRLFK NLPVRKQFYS TAKKCKDEIK KIQDLLMSFG 180
ILKPDLRIVF VHNKAVIWQK SRVSDHKMAL MSVLGTAVMN NMESFQYHSE ESQIYLSGFL 240
PKCDADHSFT SLSTPERSFI FINSRPVHQK DILKLIRHHY NLKCLKESTR LYPVFFLKID 300
VPTADVDVNL TPDKSQVLLQ NKESVLIALE NLMTTCYGPL PSTNSYENNK TDVSAADIVL 360
SKTAETDVLF NKVESSGKNY SNVDTSVIPF QNDMHNDESG KNTDDCLNHQ ISIGDFGYGH 420
CSSEISNIDK NTKNAFQDIS MSNVSWENSQ TEYSKTCFIS SVKHTQSENG NKDHIDESGE 480
NEEEAGLENS SEISADEWSR GNILKNSVGE NIEPVKILVP EKSLPCKVSN NNYPIPEQMN 540
LNEDSCNKKS NVIDNKSGKV TAYDLLSNRV IKKPMSASAL FVQDHRPQFL IENPKTSLED 600
ATLQIEELWK TLSEEEKLKY EEKATKDLER YNSQMKRAIE QESQMSLKDG RKKIKPTSAW 660
NLAQKHKLKT SLSNQPKLDE LLQSQIEKRR SQNIKMVQIP FSMKNLKINF KKQNKVDLEE 720
KDEPCLIHNL RFPDAWLMTS KTEVMLLNPY RVEEALLFKR LLENHKLPAE PLEKPIMLTE 780
SLFNGSHYLD VLYKMTADDQ RYSGSTYLSD PRLTANGFKI KLIPGVSITE NYLEIEGMAN 840
CLPFYGVADL KEILNAILNR NAKEVYECRP RKVISYLEGE AVRLSRQLPM YLSKEDIQDI 900
IYRMKHQFGN EIKECVHGRP FFHHLTYLPE TT 932
```

PMS1 (human) (SEQ ID NO:10)

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```

MSH2 (human) (SEQ ID NO:11)

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MAVQPKETLQ LESAAEVGFV RFFQGMPEKP TTTVRLFDRG DFYTAHGEDA LLAAREVFKT 60
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YKASPGNLSQ FEDILFGNND MSASIGVVGV KMSAVDGQRQ VGVGYVDSIQ RKLGLCEFPD 180
NDQFSNLEAL LIQIGPKECV LPGGETAGDM GKLRQIIQRG GILITERKKA DFSTKDIYQD 240
LNRLLKGKKG EQMNSAVLPE MENQVAVSSL SAVIKFLELL SDDSNFGQFE LTTFDFSQYM 300
KLDIAAVRAL NLFQGSVEDT TGSQSLAALL NKCKTPQGQR LVNQWIKQPL MDKNRIEERL 360
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```
NLVEAFVEDA ELRQTLQEDL LRRFPDLNRL AKKFQRQAAN LQDCYRLYQG INQLPNVIQA 420
LEKHEGKHQK LLLAVFVTPL TDLRSDFSKF QEMIETTLDM DQVENHEFLV KPSFDPNLSE 480
LREIMNDLEK KMQSTLISAA RDLGLDPGKQ IKLDSSAQFG YYFRVTCKEE KVLRNNKNFS 540
TVDIQKNGVK FTNSKLTSLN EEYTKNKTEY EEAQDAIVKE IVNISSGYVE PMQTLNDVLA 600
QLDAVVSFAH VSNGAPVPYV RPAILEKGQG RIILKASRHA CVEVQDEIAF IPNDVYFEKD 660
KQMFHIITGP NMGGKSTYIR QTGVIVLMAQ IGCFVPCESA EVSIVDCILA RVGAGDSQLK 720
GVSTFMAEML ETASILRSAT KDSLIIIDEL GRGTSTYDGF GLAWAISEYI ATKIGAFCMF 780
ATHFHELTAL ANQIPTVNNL HVTALTTEET LTMLYQVKKG VCDQSFGIHV AELANFPKHV 840
IECAKQKALE LEEFQYIGES QGYDIMEPAA KKCYLEREQG EKIIQEFLSK VKQMPFTEMS 900
EENITIKLKQ LKAEVIAKNN SFVNEIISRI KVTT                             934
```

MSH2 (human cDNA) (SEQ ID NO:12)

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gtgcagccag agatcttggc ttggaccctg gcaaacagat taaactggat tccagtgcac 1620
agtttggata ttactttcgt gtaacctgta aggaagaaaa agtccttcgt aacaataaaa 1680
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ttaaagaaat tgtcaatatt tcttcaggct atgtagaacc aatgcagaca ctcaatgatg 1860
tgttagctca gctagatgct gttgtcagct ttgctcacgt gtcaaatgga gcacctgttc 1920
catatgtacg accagccatt ttggagaaag gacaaggaag aattatatta aaagcatcca 1980
ggcatgcttg tgttgaagtt caagatgaaa ttgcatttat tcctaatgac gtatactttg 2040
aaaaagataa acagatgttc cacatcatta ctggccccaa tatgggaggt aaatcaacat 2100
atattcgaca aactggggtg atagtactca tggcccaaat tgggtgtttt gtgccatgtg 2160
agtcagcaga agtgtccatt gtggactgca tcttagcccg agtaggggct ggtgacagtc 2220
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ctgcaaccaa agattcatta ataatcatag atgaattggg aagaggaact tctacctacg 2340
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gcatgtttgc aacccatttt catgaactta ctgccttggc caatcagata ccaactgtta 2460
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ataaataaaa tcatgtagtt tgtgg                                       3145
```

MLH1 (human) (SEQ ID NO:13)

MSFVAGVIRR LDETVVNRIA AGEVIQRPAN AIKEMIENCL DAKSTSIQVI VKEGGLKLIQ 60
IQDNGTGIRK EDLDIVCERF TTSKLQSFED LASISTYGFR GEALASISHV AHVTITTKTA 120
DGKCAYRASY SDGKLKAPPK PCAGNQGTQI TVEDLFYNIA TRRKALKNPS EEYGKILEVV 180
GRYSVHNAGI SFSVKKQGET VADVRTLPNA STVDNIRSIF GNAVSRELIE IGCEDKTLAF 240
KMNGYISNAN YSVKKCIFLL FINHRLVEST SLRKAIETVY AAYLPKNTHP FLYLSLEISP 300
QNVDVNVHPT KHEVHFLHEE SILERVQQHI ESKLLGSNSS RMYFTQTLLP GLAGPSGEMV 360
KSTTSLTSSS TSGSSDKVYA HQMVRTDSRE QKLDAFLQPL SKPLSSQPQA IVTEDKTDIS 420
SGRARQQDEE MLELPAPAEV AAKNQSLEGD TTKGTSEMSE KRGPTSSNPR KRHREDSDVE 480
MVEDDSRKEM TAACTPRRRI INLTSVLSLQ EEINEQGHEV LREMLHNHSF VGCVNPQWAL 540
AQHQTKLYLL NTTKLSEELF YQILIYDFAN FGVLRLSEPA PLFDLAMLAL DSPESGWTEE 600
DGPKEGLAEY IVEFLKKKAE MLADYFSLEI DEEGNLIGLP LLIDNYVPPL EGLPIFILRL 660
ATEVNWDEEK ECFESLSKEC AMFYSIRKQY ISEESTLSGQ QSEVPGSIPN SWKWTVEHIV 720
YKALRSHILP PKHFTEDGNI LQLANLPDLY KVFERC 756

MLH1 (human) (SEQ ID NO:14)

cttggctctt ctggcgccaa aatgtcgttc gtggcagggg ttattcggcg gctggacgag 60
acagtggtga accgcatcgc ggcgggggaa gttatccagc ggccagctaa tgctatcaaa 120
gagatgattg agaactgttt agatgcaaaa tccacaagta ttcaagtgat tgttaaagag 180
ggaggcctga agttgattca gatccaagac aatggcaccg ggatcaggaa agaagatctg 240
gatattgtat gtgaaaggtt cactactagt aaactgcagt cctttgagga tttagccagt 300
atttctacct atggctttcg aggtgaggct ttggccagca taagccatgt ggctcatgtt 360
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aaactgaaag cccctcctaa accatgtgct ggcaatcaag ggacccagat cacggtggag 480
gacctttttt acaacatagc cacgaggaga aaagctttaa aaaatccaag tgaagaatat 540
gggaaaattt tggaagttgt tggcaggtat tcagtacaca atgcaggcat tagtttctca 600
gttaaaaaac aaggagagac agtagctgat gttaggacac tacccaatgc ctcaaccgtg 660
gacaatattc gctccatctt tggaaatgct gttagtcgag aactgataga aattggatgt 720
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cctatcttca ttcttcgact agccactgag gtgaattggg acgaagaaaa ggaatgtttt 2040
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gagtcgaccc tctcaggcca gcagagtgaa gtgcctggct ccattccaaa ctcctggaag 2160
tggactgtgg aacacattgt ctataaagcc ttgcgctcac acattctgcc tcctaaacat 2220
ttcacagaag atggaaatat cctgcagctt gctaacctgc ctgatctata caaagtcttt 2280
gagaggtgtt aaatatggtt atttatgcac tgtgggatgt gttcttcttt ctctgtattc 2340
cgatacaaag tgttgtatca aagtgtgata tacaaagtgt accaacataa gtgttggtag 2400
cacttaagac ttatacttgc cttctgatag tattccttta tacacagtgg attgattata 2460
aataaataga tgtgtcttaa cata 2484

hPMS2-134 (human) (SEQ ID NO:15)

```
MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENSLDAG ATNIDLKLKD 60
YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
TISTCHASAK VGT                                                    133
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hPMS2-134 (human cDNA) (SEQ ID NO:16)

```
cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120
ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180
aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
acttga                                                            426
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The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1: Stable expression of dominant negative MMR genes in hybridoma cells

It has been previously shown by Nicolaides et al. (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641) that the expression of a dominant negative allele in an otherwise MMR proficient cell could render these host cells MMR deficient. The creation of MMR deficient cells can lead to the generation of genetic alterations

throughout the entire genome of a host organisms offspring, yielding a population of genetically altered offspring or siblings that may produce biochemicals with altered properties. This patent application teaches of the use of dominant negative MMR genes in antibody-producing cells, including but not limited to rodent hybridomas, human hybridomas, chimeric rodent cells producing human immunoglobulin gene products, human cells expressing immunoglobulin genes, mammalian cells producing single chain antibodies, and prokaryotic cells producing mammalian immunoglobulin genes or chimeric immunoglobulin molecules such as those contained within single-chain antibodies. The cell expression systems described above that are used to produce antibodies are well known by those skilled in the art of antibody therapeutics.

To demonstrate the ability to create MMR defective hybridomas using dominant negative alleles of MMR genes, we first transfected a mouse hybridoma cell line that is known to produce and antibody directed against the human IgE protein with an expression vector containing the human PMS2 (cell line referred to as HBPMS2), the previously published dominant negative PMS2 mutant referred herein as PMS134 (cell line referred to as HB134), or with no insert (cell line referred to as HBvec). The results showed that the PMS134 mutant could indeed exert a robust dominant negative effect, resulting in biochemical and genetic manifestations of MMR deficiency. Unexpectedly was the finding that the full length PMS2 also resulted in a lower MMR activity while no effect was seen in cells containing the empty vector. A brief description of the methods is provided below.

The MMR proficient mouse H36 hybridoma cell line was transfected with various *hPMS2* expression plasmids plus reporter constructs for assessing MMR activity. The MMR genes were cloned into the pEF expression vector, which contains the elongation factor promoter upstream of the cloning site followed by a mammalian polyadenylation signal. This vector also contains the NEOr gene that allows for selection of cells retaining this plasmid. Briefly, cells were transfected with 1 μg of each vector using polyliposomes following the manufacturer's protocol (Life Technologies). Cells were then selected in 0.5 mg/ml of G418 for 10 days and G418 resistant cells were pooled together to analyze for gene expression. The pEF construct contains an intron that separates the exon 1 of the EF gene from exon 2, which is juxtaposed to the 5' end of the polylinker cloning site. This allows for a rapid reverse transcriptase polymerase chain reaction (RT-PCR) screen for cells expressing the spliced

products. At day 17, 100,000 cells were isolated and their RNA extracted using the trizol method as previously described (Nicolaides N.C., Kinzler, K.W., and Vogelstein, B. (1995) Analysis of the 5' region of PMS2 reveals heterogeneous transcripts and a novel overlapping gene. *Genomics* 29:329-334). RNAs were reverse transcribed using Superscript II (Life Technologies) and PCR amplified using a sense primer located in exon 1 of the EF gene (5'-ttt cgc aac ggg ttt gcc g-3') and an antisense primer (5'-gtt tca gag tta agc ctt cg-3') centered at nt 283 of the published human PMS2 cDNA, which will detect both the full length as well as the PMS134 gene expression. Reactions were carried out using buffers and conditions as previously described (Nicolaides, N.C., *et al.* (1995) Genomic organization of the human PMS2 gene family. *Genomics* 30:195-206), using the following amplification parameters: 94°C for 30 sec, 52°C for 2 min, 72°C for 2 min, for 30 cycles. Reactions were analyzed on agarose gels. Figure 1 shows a representative example of PMS expression in stably transduced H36 cells.

Expression of the protein encoded by these genes were confirmed via western blot using a polyclonal antibody directed to the first 20 amino acids located in the N-terminus of the protein following the procedures previously described (data not shown) (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641.

EXAMPLE 2: hPMS134 Causes a Defect in MMR Activity and hypermutability in hybridoma cells

A hallmark of MMR deficiency is the generation of unstable microsatellite repeats in the genome of host cells. This phenotype is referred to as microsatellite instability (MI) (Modrich, P. (1994) Mismatch repair, genetic stability, and cancer *Science* 266:1959-1960; Palombo, F., *et al.* (1994) Mismatch repair and cancer *Nature* 36:417). MI consists of deletions and/or insertions within repetitive mono-, di- and/or tri nucleotide repetitive sequences throughout the entire genome of a host cell. Extensive genetic analysis eukaryotic cells have found that the only biochemical defect that is capable of producing MI is defective MMR (Strand, M., *et al.* (1993) Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair *Nature* 365:274-276; Perucho, M. (1996) Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684; Eshleman J.R., and Markowitz, S.D. (1996) Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.* 5:1489-494). In light of this unique feature

that defective MMR has on promoting MI, it is now used as a biochemical marker to survey for lack of MMR activity within host cells (Perucho, M. (1996) Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684; Eshleman J.R., and Markowitz, S.D. (1996) Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.* 5:1489-494; Liu, T., *et al.* (2000) Microsatellite instability as a predictor of a mutation in a DNA mismatch repair gene in familial colorectal cancer *Genes Chromosomes Cancer* 27:17-25).

A method used to detect MMR deficiency in eukaryotic cells is to employ a reporter gene that has a polynucleotide repeat inserted within the coding region that disrupts its reading frame due to a frame shift. In the case where MMR is defective, the reporter gene will acquire random mutations (i.e. insertions and/or deletions) within the polynucleotide repeat yielding clones that contain a reporter with an open reading frame. We have employed the use of an MMR-sensitive reporter gene to measure for MMR activity in HBvec, HBPMS2, and HBPMS134 cells. The reporter construct used the pCAR-OF, which contains a hygromycin resistance (HYG) gene plus a β-galactosidase gene containing a 29 bp out-of-frame poly-CA tract at the 5' end of its coding region. The pCAR-OF reporter would not generate β -galactosidase activity unless a frame-restoring mutation (*i.e.*, insertion or deletion) arose following transfection. HBvec, HBPMS2, and HB134 cells were each transfected with pCAR-OF vector in duplicate reactions following the protocol described in Example 1. Cells were selected in 0.5 mg/ml G418 and 0.5mg/ml HYG to select for cells retaining both the MMR effector and the pCAR-OF reporter plasmids. All cultures transfected with the pCAR vector resulted in a similar number of HYG/G418 resistant cells. Cultures were then expanded and tested for β-galactosidase activity *in situ* as well as by biochemical analysis of cell extracts. For *in situ* analysis, 100,000 cells were harvested and fixed in 1% gluteraldehyde, washed in phosphate buffered saline solution and incubated in 1 ml of X-gal substrate solution [0.15 M NaCl, 1 mM $MgCl_2$, 3.3 mM $K_4Fe(CN)_6$, 3.3 mM $K_3Fe(CN)_6$, 0.2% X-Gal] in 24 well plates for 2 hours at 37°C. Reactions were stopped in 500 mM sodium bicarbonate solution and transferred to microscope slides for analysis. Three fields of 200 cells each were counted for blue (β-galactosidase positive cells) or white (β-galactosidase negative cells) to assess for MMR inactivation. Table 1 shows the results from these studies. While no β-galactosidase positive cells were observed in HBvec cells, 10% of the cells per

field were β-galactosidase positive in HB134 cultures and 2% of the cells per field were β-galactosidase positive in HBPMS2 cultures.

Cell extracts were prepared from the above cultures to measure β-galactosidase using a quantitative biochemical assay as previously described (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641; Nicolaides, N.C., *et al.* (1992) The Jun family members, c-JUN and JUND, transactivate the human *c-myb* promoter via an Ap1 like element. *J. Biol. Chem.* 267:19665-19672). Briefly, 100,000 cells were collected, centrifuged and resuspended in 200 μls of 0.25M Tris, pH 8.0. Cells were lysed by freeze/thawing three times and supernatants collected after microfugation at 14,000 rpms to remove cell debris. Protein content was determined by spectrophotometric analysis at OD^{280}. For biochemical assays, 20 μg of protein was added to buffer containing 45 mM 2-mercaptoethanol, 1mM $MgCl_2$, 0.1 M $NaPO_4$ and 0.6 mg/ml Chlorophenol red-β-D-galactopyranoside (CPRG, Boehringer Mannheim). Reactions were incubated for 1 hour, terminated by the addition of 0.5 M Na_2CO_3, and analyzed by spectrophotometry at 576 nm. H36 cell lysates were used to subtract out background. Figure 2 shows the β-galactosidase activity in extracts from the various cell lines. As shown, the HB134 cells produced the highest amount of β-galactosidase, while no activity was found in the HBvec cells containing the pCAR-OF. These data demonstrate the ability to generate MMR defective hybridoma cells using dominant negative MMR gene alleles.

Table 1. β-galactosidase expression of HBvec, HBPMS2 and HB134 cells transfected with pCAR-OF reporter vectors. Cells were transfected with the pCAR-OF β-galactosidase reporter plasmid. Transfected cells were selected in hygromycin and G418, expanded and stained with X-gal solution to measure for β-galactosidase activity (blue colored cells). 3 fields of 200 cells each were analyzed by microscopy. The results below represent the mean +/- standard deviation of these experiments.

produced by each clone. Reactions are stopped by adding 50 μls of 500mM sodium bicarbonate and analyzed by OD at 415nm using a BioRad plate reader. Clones exhibiting an enhanced signal over background cells (H36 control cells) are then isolated and expanded into 10 ml cultures for additional characterization and confirmation of ELISA data in triplicate experiments. ELISAs are also performed on conditioned (CM) from the same clones to measure total Ig production within the conditioned medium of each well. Clones that produce an increased ELISA signal and have increased antibody levels are then further analyzed for variants that over-express and/or over-secrete antibodies as described in Example 4. Analysis of five 96-well plates each from HBvec or HB134 cells have found that a significant number of clones with a higher Optical Density (OD) value is observed in the MMR-defective HB134 cells as compared to the HBvec controls. Figure 4 shows a representative example of HB134 clones producing antibodies that bind to specific antigen (in this case IgE) with a higher affinity. Figure 4 provides raw data from the analysis of 96 wells of HBvec (left graph) or HB134 (right graph) which shows 2 clones from the HB134 plate to have a higher OD reading due to 1) genetic alteration of the antibody variable domain that leads to an increased binding to IgE antigen, or 2) genetic alteration of a cell host that leads to over-production/secretion of the antibody molecule. Anti-Ig ELISA found thatthe two clones, shown in figure 4 have Ig levels within their CM similar to the surrounding wells exhibiting ower OD values. These data suggest that a genetic alteration occurred within the antigen binding domain of the antibody which in turn allows for higher binding to antigen.

Clones that produced higher OD values as determined by ELISA were further analyzed at the genetic level to confirm that mutations within the light or heavy chain variable region have occurred that lead to a higher binding affinity hence yielding to a stronger ELISA signal. Briefly, 100,000 cells are harvested and extracted for RNA using the Triazol method as described above. RNAs are reverse transcribed using Superscript II as suggested by the manufacturer (Life Technology) and PCR amplified for the antigen binding sites contained within the variable light and heavy chains. Because of the heterogeneous nature of these genes, the following degenerate primers are used to amplify light and heavy chain alleles from the parent H36 strain.

Light chain sense: 5'-GGA TTT TCA GGT GCA GAT TTT CAG-3' (SEQ ID NO:1)

Light chain antisense: 5'-ACT GGA TGG TGG GAA GAT GGA-3' (SEQ ID NO:2)

Heavy chain sense: 5'-A(G/T) GTN (A/C)AG CTN CAG (C/G)AG TC-3' (SEQ ID NO:3)

Heavy chain antisense: 5'-TNC CTT G(A/G)C CCC AGT A(G/A)(A/T)C-3' (SEQ ID NO:4)

PCR reactions using degenerate oligonucleotides are carried out at 94°C for 30 sec, 52°C for 1 min, and 72°C for 1 min for 35 cycles. Products are analyzed on agarose gels. Products of the expected molecular weights are purified from the gels by Gene Clean (Bio 101), cloned into T-tailed vectors, and sequenced to identify the wild type sequence of the variable light and heavy chains. Once the wild type sequence has been determined, non-degenerate primers were made for RT-PCR amplification of positive HB134 clones. Both the light and heavy chains were amplified, gel purified and sequenced using the corresponding sense and antisense primers. The sequencing of RT-PCR products gives representative sequence data of the endogenous immunoglobulin gene and not due to PCR induced mutations. Sequences from clones were then compared to the wild type sequence for sequence comparison. An example of the ability to create *in vivo* mutations within an immunoglobulin light or heavy chain is shown in figure 5, where HB134 clone92 was identified by ELISA to have an increased signal for hIgE. The light chain was amplified using specific sense and antisense primers. The light chain was RT-PCR amplified and the resulting product was purified and analyzed on an automated ABI377 sequencer. As shown in clone A, a residue –4 upstream of the CDR region 3 had a genetic change from ACT to TCT, which results in a Thr to Ser change within the framework region just preceding the CDR#3. In clone B, a residue –6 upstream of the CDR region had a genetic change from CCC to CTC, which reslts in a Pro to His change within framework region preceeding CDR#2.

The ability to generate random mutations in immunoglobulin genes or chimeric immunoglobulin genes is not limited to hybridomas. Nicolaides et al. (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641) has previously shown the ability to generate hypermutable hamster cells and produce mutations within an endogenous gene. A common method for producing humanized antibodies is to graft CDR sequences from a MAb

(produced by immunizing a rodent host) onto a human Ig backbone, and transfection of the chimeric genes into Chinese Hamster Ovary (CHO) cells whih in turn produce a functional Ab that is secreted by the CHO cells (Shields, R.L., *et al.* (1995) Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int. Arch. Allergy Immunol.* 107:412-413). The methods described within this application are also useful for generating genetic alterations within Ig genes or chimeric Igs transfected within host cells such as rodent cell lines, plants, yeast and prokaryotes (Frigerio L, *et al.* (2000) Assembly, secretion, and vacuolar delivery of a hybrid immunoglobulin in plants. *Plant Physiol.* 123:1483-1494).

These data demonstrate the ability to generate hypermutable hybridomas, or other Ig producing host cells that can be grown and selected, to identify structurally altered immunoglobulins yielding antibodies with enhanced biochemical properties, including but not limited to increased antigen binding affinity. Moreover, hypermutable clones that contain missense mutations within the immunoglobulin gene that result in an amino acid change or changes can be then further characterized for *in vivo* stability, antigen clearance, on-off binding to antigens, etc. Clones can also be further expanded for subsequent rounds of *in vivo* mutations and can be screened using the strategy listed above.

The use of chemical mutagens to produce genetic mutations in cells or whole organisms are limited due to the toxic effects that these agents have on "normal" cells. The use of chemical mutagens such as MNU in MMR defective organisms is much more tolerable yielding to a 10 to 100 fold increase in genetic mutation over MMR deficiency alone (Bignami M, (2000) Unmasking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res.* 462:71-82). This strategy allows for the use of chemical mutagens to be used in MMR-defective Ab producing cells as a method for increasing additional mutations within immunoglobulin genes or chimeras that may yield functional Abs with altered biochemical properties such as enhanced binding affinity to antigen, etc.

Example 4: Generation of antibody producing cells with enhanced antibody production

Analysis of clones from H36 and HB134 following the screening strategy listed above hasidentified a significant number of clones that produce enhanced amounts of antibody into the medium. While a subset of these clones gave higher Ig binding data as determined by ELISA as a consequence of mutations within the antigen binding domains contained in the variable regions, others were found to contain "enhanced" antibody production. A summary of the clones producing enhanced amounts of secreted MAb is shown in TABLE 2, where a significant number of clones from HB134 cells were found to produce enhanced Ab production within the conditioned medium as compared to H36 control cells.

TABLE 2. Generation of hybridoma cells producing high levels of antibody. HB134 clones were assayed by ELISA for elevated Ig levels. Analysis of 480 clones showed that a significant number of clones had elevated MAb product levels in their CM. Quantification showed that several of these clones produced greater than 500ngs/ml of MAb due to either enhanced expression and/or secretion as compared to clones from the H36 cell line.

Table 2. Production of MAb in CM from H36 and HB134 clones.

Cell Line	% clones > 400 ng/ml	% clones >500 ng/ml
H36	1/480 = 0.2%	0/480 = 0%
HB134	50/480 = 10%	8/480 = 1.7%

Cellular analysis of HB134 clones with higher MAb levels within the conditioned medium (CM) were analyzed to determine if the increased production was simply due to genetic alterations at the Ig locus that may lead to over-expression of the polypeptides forming the antibody, or due to enhanced secretion due to a genetic alteration affecting secretory pathway mechanisms. To address this issue, we expanded three HB134 clones that had increased levels of antibody within their CM. 10,000 cells were prepared for western blot analysis to assay for intracellular steady state Ig protein levels (Figure 6). In addition, H36 cells were used as a standard reference (Lane 2) and a rodent fibroblast (Lane 1) was used as an Ig negative control. Briefly, cells were pelleted by centrifugation and lysed directly in 300 μl of SDS lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 10% glycerol,

0.1 M 2-mercaptoethanol, 0.001% bromophenol blue) and boiled for 5 minutes. Lysate proteins were separated by electrophoresis on 4-12% NuPAGE gels (for analysis of Ig heavy chain. Gels were electroblotted onto Immobilon-P (Millipore) in 48 mM Tris base, 40 mM glycine, 0.0375% SDS, 20% methanol and blocked at room temperature for 1 hour in Tris-buffered saline (TBS) plus 0.05% Tween-20 and 5% condensed milk. Filters were probed with a 1:10,000 dilution of sheep anti-mouse horseradish peroxidase conjugated monoclonal antibody in TBS buffer and detected by chemiluminescence using Supersignal substrate (Pierce). Experiments were repeated in duplicates to ensure reproducibility. Figure 6 shows a representative analysis where a subset of clones had enhanced Ig production which accounted for increased Ab production (Lane 5) while others had a similar steady state level as the control sample, yet had higher levels of Ab within the CM. These data suggest a mechanism whereby a subset of HB134 clones contained a genetic alteration that in turn produces elevated secretion of antibody.

The use of chemical mutagens to produce genetic mutations in cells or whole organisms are limited due to the toxic effects that these agents have on "normal" cells. The use of chemical mutagens such as MNU in MMR defective organisms is much more tolerable yielding to a 10 to 100 fold increase in genetic mutation over MMR deficiency alone (Bignami M, (2000) Unmasking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res.* 462:71-82). This strategy allows for the use of chemical mutagens to be used in MMR-defective Ab producing cells as a method for increasing additional mutations within immunoglobulin genes or chimeras that may yield functional Abs with altered biochemical properties such as enhanced binding affinity to antigen, etc.

Example 5: establishment of genetic stability in hybridoma cells with new output trait.

The initial steps of MMR are dependent on two protein complexes, called MutSα and MutLα (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641). Dominant negative MMR alleles are able to perturb the formation of these complexes with downstream biochemicals involved in the excision and polymerization of nucleotides comprising the "corrected" nucleotides. Examples from this application show the ability of a truncated MMR allele (PMS134) as well as a full length human PMS2 when expressed in

a hybridoma cell line is capable of blocking MMR resulting in a hypermutable cell line that gains genetic alterations throughout its entire genome per cell division. Once a cell line is produced that contains genetic alterations within genes encoding for an antibody, a single chain antibody, over expression of immunoglobulin genes and/or enhanced secretion of antibody, it is desirable to restore the genomic integrity of the cell host. This can be achieved by the use of inducible vectors whereby dominant negative MMR genes are cloned into such vectors, introduced into Ab producing cells and the cells are cultured in the presence of inducer molecules and/or conditions. Inducible vectors include but are not limited to chemical regulated promoters such as the steroid inducible MMTV, tetracycline regulated promoters, temperature sensitive MMR gene alleles, and temperature sensitive promoters.

The results described above lead to several conclusions. First, expression of hPMS2 and PMS134 results in an increase in microsatellite instability in hybridoma cells. That this elevated microsatellite instability is due to MMR deficiency was proven by evaluation of extracts from stably transduced cells. The expression of PMS134 results in a polar defect in MMR, which was only observed using heteroduplexes designed to test repair from the 5' direction (no significant defect in repair from the 3' direction was observed in the same extracts) (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641). Interestingly, cells deficient in hMLH1 also have a polar defect in MMR, but in this case preferentially affecting repair from the 3' direction (Drummond, J.T, *et al.* (1996) Cisplatin and adriamycin resistance are associated with MutLa and mismatch repair deficiency in an ovarian tumor cell line. *J. Biol. Chem.* 271:9645-19648). It is known from previous studies in both prokaryotes and eukaryotes that the separate enzymatic components mediate repair from the two different directions. Our results, in combination with those of Drummond *et al.*(Shields, R.L., *et al.* (1995) Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int. Arch Allergy Immunol.* 107:412-413), strongly suggest a model in which 5' repair is primarily dependent on hPMS2 while 3' repair is primarily dependent on hMLH1. It is easy to envision how the dimeric complex between PMS2 and MLH1 might set up this directionality. The combined results also demonstrate that a defect in directional MMR is sufficient to produce a MMR defective phenotype and suggests that any MMR gene allele is useful to produce genetically altered hybridoma cells, or a cell line that is producing Ig gene products. Moreover, the use of

such MMR alleles will be useful for generating genetically altered Ig polypeptides with altered biochemical properties as well as cell hosts that produce enhanced amounts of antibody molecules.

Another method that is taught in this application is that ANY method used to block MMR can be performed to generate hypermutablility in an antibody-producing cell that can lead to genetically altered antibodies with enhanced biochemical features such as but not limited to increased antigen binding, enhanced pharmacokinetic profiles, etc. These processes can also to be used to generate antibody producer cells that have increased Ig expression as shown in Example 4, figure 6 and/or increased antibody secretion as shown in Table 2.

In addition, we demonstrate the utility of blocking MMR in antibody producing cells to increase genetic alterations within Ig genes that may lead to altered biochemical features such as, but not limited to, increased antigen binding affinities (Figure 5A and 5B). The blockade of MMR in such cells can be through the use of dominant negative MMR gene alleles from any species including bacteria, yeast, protozoa, insects, rodents, primates, mammalian cells, and man. Blockade of MMR can also be generated through the use of antisense RNA or deoxynucleotides directed to any of the genes involved in the MMR biochemical pathway. Blockade of MMR can be through the use of polypeptides that interfere with subunits of the MMR complex including but not limited to antibodies. Finally, the blockade of MMR may be through the use chemicals such as but not limited to nonhydrolyzable ATP analogs, which have been shown to block MMR (Galio, L, *et al.* (1999) ATP hydrolysis-dependent formation of a dynamic ternary nucleoprotein complex with MutS and MutL. *Nucl. Acids Res.* 27:2325-23231).

WE CLAIM:

1. A method for making a hypermutable, antibody producing cell, comprising introducing into a cell capable of producing antibodies a polynucleotide comprising a dominant negative allele of a mismatch repair gene.
2. The method of claim 1 wherein said polynucleotide is introduced by transfection of a suspension of cells *in vitro*.
3. The method of claim 1 wherein said mismatch repair gene is *PMS2.*
4. The method of claim 1 wherein said mismatch repair gene is human *PMS2.*
5. The method of claim 1 wherein said mismatch repair gene is *MLH1.*
6. The method of claim 1 wherein said mismatch repair gene is *PMS1.*
7. The method of claim 1 wherein said mismatch repair gene is *MSH2.*
8. The method of claim 1 wherein said mismatch repair gene is *MSH2.*
9. The method of claim 4 wherein said allele comprises a truncation mutation.
10. The method of claim 4 wherein said allele comprises a truncation mutation at codon 134.
11. The method of claim 10 wherein said truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2*.
12. The method of claim 1 wherein said polynucleotide is introduced into a fertilized egg of an animal.
13. The method of claim 12 wherein the fertilized egg is subsequently implanted into a pseudo-pregnant female whereby the fertilized egg develops into a mature transgenic animal.
14. The method of claim 12 wherein said mismatch repair gene is *PMS2.*
15. The method of claim 12 wherein said mismatch repair gene is human *PMS2.*
16. The method of claim 12 wherein said mismatch repair gene is human *MLH1.*
17. The method of claim 12 wherein said mismatch repair gene is human *PMS1.*
18. The method of claim 11 wherein said mismatch repair gene is a human *mutL* homolog.
19. The method of claim 15 wherein said allele comprises a truncation mutation.
20. The method of claim 15 wherein said allele comprises a truncation mutation at codon 134.
21. The method of claim 19 wherein said truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2*.

22. The method of claim 1 wherein said capability is due to the co-introduction of an immunoglobulin gene into said cell.

23. A homogeneous culture of hypermutable, mammalian cells wherein said cells comprise a dominant negative allele of a mismatch repair gene.

24. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *PMS2*.

25. The culture of hypermutable, mammalian cells of claim 24 wherein the mismatch repair gene is human *PMS2*.

26. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *MLH1*.

27. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *PMS1*.

28. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is a human *mutL* homolog.

29. The culture of hypermutable, mammalian cells of claim 23 wherein the cells express a protein consisting of the first 133 amino acids of hPMS2.

30. A method for generating a mutation in a gene affecting antibody production in an antibody-producing cell comprising:

growing a said cell comprising said gene and a dominant negative allele of a mismatch repair gene; and

testing the cell to determine whether said gene of interest harbors a mutation.

31. The method of claim 30 wherein the step of testing comprises analyzing a nucleotide sequence of said gene.

32. The method of claim 30 wherein the step of testing comprises analyzing mRNA transcribed from said gene.

33. The method of claim 30 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

34. The method of claim 30 wherein the step of testing comprises analyzing the phenotype of said gene.

35. The method of claim 30 wherein the step of testing comprises analyzing the binding activity of an antibody.

36. A method wherein a mammalian cell is made MMR defective by the process of introducing a polynucleotide comprising an antisense oligonucleotide targeted against an allele of a mismatch repair gene into a mammalian cell, whereby the cell becomes hypermutable.

37. The method of claim 36 wherein the step of testing comprises analyzing a nucleotide sequence of said gene.

38. The method of claim 36 wherein the step of testing comprises analyzing mRNA transcribed from said gene.

39. The method of claim 36 wherein the step of testing comprises analyzing a protein encoded by said gene.

40. The method of claim 36 wherein the step of testing comprises analyzing the phenotype of said gene.

41. The method of claim 36 wherein the step of testing comprises analyzing the binding activity of an antibody.

42. A method for generating a mutation in a gene affecting antibody production in an antibody-producing cell comprising:

growing said cell comprising said gene and a polynucleotide encoding a dominant negative allele of a mismatch repair gene; and

testing said cell to determine whether said cell harbors at least one mutation in said gene yielding to a new biochemical feature to the product of said gene, wherein said new biochemical feature is selected from the group consisting of over-expression of said product, enhanced secretion of said product, enhanced affinity of said product for antigen, and combinations thereof.

43. The method of claim 42 wherein the step of testing comprises analyzing the steady state expression of the immunoglobulin gene of said cell.

44. The method of claim 42 wherein the step of testing comprises analyzing steady state mRNA transcribed from the immunoglobulin gene of said cell.

45. The method of claim 42 wherein the step of testing comprises analyzing the amount of secreted protein encoded by the immunoglobulin gene of said cell.

46. The method of claim 36 wherein the cell is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a cell in the presence of DNA mutagens.

47. The method of claim 46 wherein the step of testing comprises analyzing a nucleotide sequence of an immunoglobulin gene of said cell.
48. The method of claim 46 wherein the step of testing comprises analyzing mRNA transcribed from the immunoglobulin gene of said cell.
49. The method of claim 46 wherein the step of testing comprises analyzing the immunoglobulin protein encoded by said gene.
50. The method of claim 46 wherein the step of testing comprises analyzing the biochemical activity of the protein encoded by said gene.
51. A hypermutable transgenic mammalian cell made by the method of claim 42.
52. The transgenic mammalian cell of claim 51 wherein said cell is from primate.
53. The transgenic mammalian cell of claim 51 wherein said cell is from rodent.
54. The transgenic mammalian cell of claim 51 wherein said cell is from human.
55. The transgenic mammalian cell of claim 51 wherein said cell is eukaryotic.
56. The transgenic mammalian cell of claim 51 wherein said cell is prokaryotic
57. A method of reversibly altering the hypermutability of an antibody producing cell comprising introducing an inducible vector into a cell, wherein said inducible vector comprises a dominant negative allele of a mismatch repair gene operably linked to an inducible promoter, and inducing said cell to express said dominant negative mismatch repair gene.
58. The method of claim 57 wherein said mismatch repair gene is *PMS2*.
59. The method of claim 58 wherein said mismatch repair gene is human *PMS2*.
60. The method of claim 57 wherein said mismatch repair gene is *MLH1*.
61. The method of claim 57 wherein said mismatch repair gene is *PMS1*.
62. The method of claim 57 wherein said mismatch repair gene is a human *mutL* homolog.
63. The method of claim 57 wherein said cell expresses a protein consisting of the first 133 amino acids of hPMS2.
64. The method of claim 57 further comprising analyzing the immunoglobulin protein expressed by said antibody producing cell.
65. The method of claim 64 further comprising ceasing induction of said cell, thereby restoring genetic stability of said cell.
66. A method of producing genetically altered antibodies comprising

transfecting a polynucleotide encoding an immunoglobulin protein into a cell, wherein said cell comprises a dominant negative mismatch repair gene;

growing said cell, thereby producing a hypermutated polynucleotide encoding a hypermutated immunoglobulin protein;

screening for a desirable property of said hypermutated immunoglobulin protein;

isolating said hypermutated polynucleotide; and

transfecting said hypermutated polynucleotide into a genetically stable cell, thereby producing a hypermutated antibody-producing, genetically stable cell.

67. The method of claim 66 wherein said mismatch repair gene is *PMS2*.

68. The method of claim 66 wherein said mismatch repair gene is human *PMS2*.

69. The method of claim 66 wherein said mismatch repair gene is *MLH1*.

70. The method of claim 66 wherein said mismatch repair gene is *PMS1*.

71. The method of claim 66 wherein said mismatch repair gene is a human *mutL* homolog.

72. The method of claim 66 wherein said cell expresses a protein consisting of the first 133 amino acids of hPMS2.

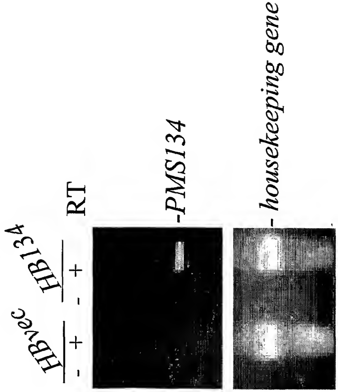
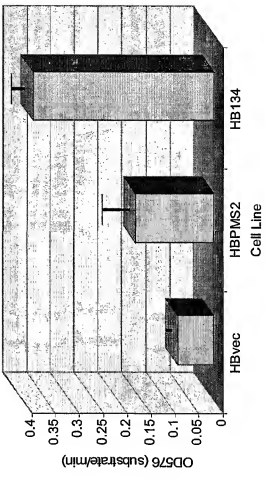


FIG. 1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0.35
0.3
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HBvec
HBPMS2
HB134
Cell Line

Fig. 2

Fig. 3

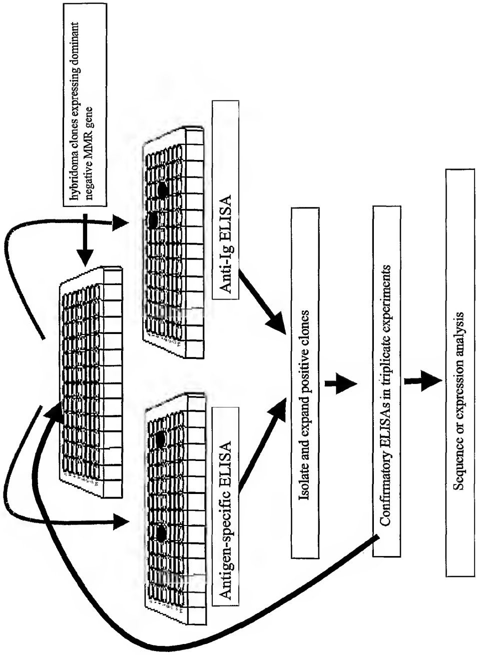
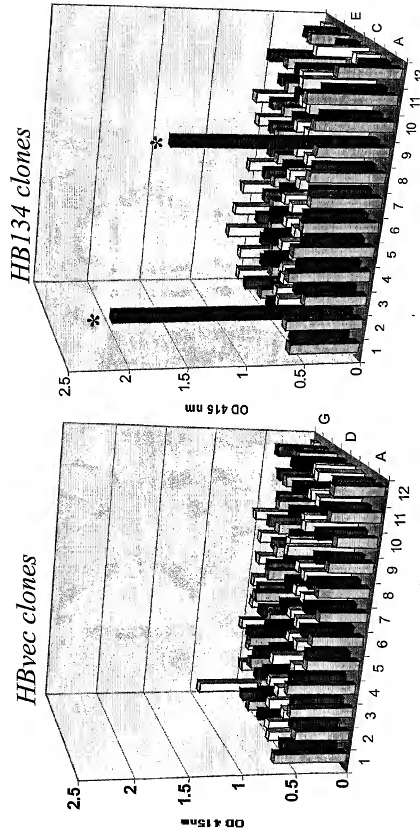
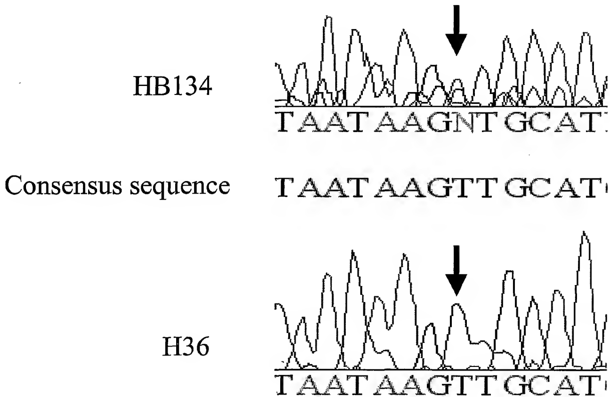

hybridoma clones expressing dominant negative MMR gene
Anti-Ig ELISA
Antigen-specific ELISA
Isolate and expand positive clones
Confirmatory ELISAs in triplicate experiments
Sequence or expression analysis

Fig.4



* = clones with a significant difference in antigen binding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Consensus sequence
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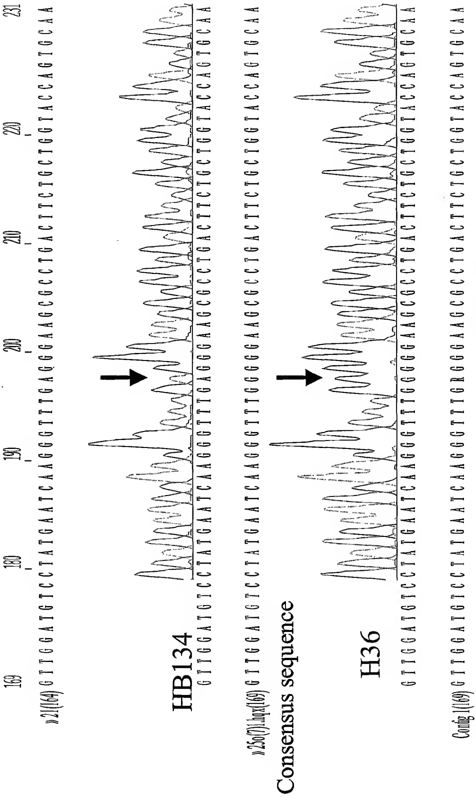
Fig. 5A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Consensus sequence
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Contig 1(169)

Fig. 5B

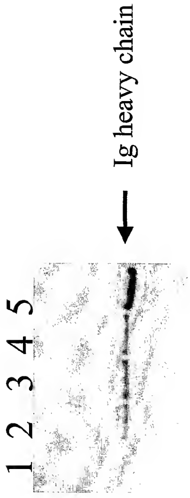

Fig. 6

SEQUENCE LISTING

<110> Nicolaides, Nicholas C
Grasso, Luigi
Sass, Philip M

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35 40 45

Ala Gly Ala Thr Thr Ile Asp Leu Arg Leu Lys Asp Tyr Gly Val Asp
50 55 60

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85 90 95

Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser

100 105 110

Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Gly Ser
115 120 125

Ala Ser Val Gly Thr Arg Leu Val Phe Asp His Asn Gly Lys Ile Thr
130 135 140

Gln Lys Thr Pro Tyr Pro Arg Pro Lys Gly Thr Thr Val Ser Val Gln
145 150 155 160

His Leu Phe Tyr Thr Leu Pro Val Arg Tyr Lys Glu Phe Gln Arg Asn
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Pro Phe Val Gln Leu Pro Pro Ser Asp Ala Val Cys Glu Glu Tyr Gly
245 250 255

Leu Ser Thr Ser Gly Arg His Lys Thr Phe Ser Thr Phe Arg Ala Ser
260 265 270

Phe His Ser Ala Arg Thr Ala Pro Gly Gly Val Gln Gln Thr Gly Ser
275 280 285

Phe Ser Ser Ser Ile Arg Gly Pro Val Thr Gln Gln Arg Ser Leu Ser
290 295 300

Leu Ser Met Arg Phe Tyr His Met Tyr Asn Arg His Gln Tyr Pro Phe
305 310 315 320

Val Val Leu Asn Val Ser Val Asp Ser Glu Cys Val Asp Ile Asn Val
325 330 335

Thr Pro Asp Lys Arg Gln Ile Leu Leu Gln Glu Glu Lys Leu Leu Leu
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Lys Leu Asn Val Asn Gln Gln Pro Leu Leu Asp Val Glu Gly Asn Leu
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Asp Asn Ser Pro Ser Leu Lys Ser Thr Ala Asp Glu Lys Arg Val Ala
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Ser Ile Ser Arg Leu Arg Glu Ala Phe Ser Leu His Pro Thr Lys Glu
420 425 430

Ile Lys Ser Arg Gly Pro Glu Thr Ala Glu Leu Thr Arg Ser Phe Pro
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450 455 460

Tyr Arg Gly Leu Arg Gly Ser Gln Asp Lys Leu Val Ser Pro Thr Asp
465 470 475 480

Ser Pro Gly Asp Cys Met Asp Arg Glu Lys Ile Glu Lys Asp Ser Gly
485 490 495

Leu Ser Ser Thr Ser Ala Gly Ser Glu Glu Glu Phe Ser Thr Pro Glu
500 505 510

Val Ala Ser Ser Phe Ser Ser Asp Tyr Asn Val Ser Ser Leu Glu Asp
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Arg Pro Ser Gln Glu Thr Ile Asn Cys Gly Asp Leu Asp Cys Arg Pro
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Pro Gly Thr Gly Gln Ser Leu Lys Pro Glu Asp His Gly Tyr Gln Cys
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Lys Ala Leu Pro Leu Ala Arg Leu Ser Pro Thr Asn Ala Lys Arg Phe
565 570 575

Lys Thr Glu Glu Arg Pro Ser Asn Val Asn Ile Ser Gln Arg Leu Pro
580 585 590

Gly Pro Gln Ser Thr Ser Ala Ala Glu Val Asp Val Ala Ile Lys Met
595 600 605

Asn Lys Arg Ile Val Leu Leu Glu Phe Ser Leu Ser Ser Leu Ala Lys

610 615 620

Arg Met Lys Gln Leu Gln His Leu Lys Ala Gln Asn Lys His Glu Leu
625 630 635 640

Ser Tyr Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu Asn Gln Ala
645 650 655

Ala Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Ser Met Phe Ala Glu
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Met Glu Ile Leu Gly Gln Phe Asn Leu Gly Phe Ile Val Thr Lys Leu
675 680 685

Lys Glu Asp Leu Phe Leu Val Asp Gln His Ala Ala Asp Glu Lys Tyr
690 695 700

Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Ala Gln Arg Leu
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Ile Thr Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu Ala Val Leu
725 730 735

Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly Phe Asp Phe Val Ile
740 745 750

Asp Glu Asp Ala Pro Val Thr Glu Arg Ala Lys Leu Ile Ser Leu Pro
755 760 765

Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Ile Asp Glu Leu Ile
770 775 780

Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro Ser Arg Val
785 790 795 800

Arg Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val Met Ile Gly
805 810 815

Thr Ala Leu Asn Ala Ser Glu Met Lys Lys Leu Ile Thr His Met Gly
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<213> Homo sapiens

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Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp
        35                  40                  45

Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp
    50                  55                  60

Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
65                  70                  75                  80

Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
                85                  90                  95

Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser
            100                 105                 110

Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Ala Ser
        115                 120                 125

Ala Lys Val Gly Thr Arg Leu Met Phe Asp His Asn Gly Lys Ile Ile
    130                 135                 140

Gln Lys Thr Pro Tyr Pro Arg Pro Arg Gly Thr Thr Val Ser Val Gln
145                 150                 155                 160

Gln Leu Phe Ser Thr Leu Pro Val Arg His Lys Glu Phe Gln Arg Asn
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Ile Lys Lys Glu Tyr Ala Lys Met Val Gln Val Leu His Ala Tyr Cys
180 185 190

Ile Ile Ser Ala Gly Ile Arg Val Ser Cys Thr Asn Gln Leu Gly Gln
195 200 205

Gly Lys Arg Gln Pro Val Val Cys Thr Gly Gly Ser Pro Ser Ile Lys
210 215 220

Glu Asn Ile Gly Ser Val Phe Gly Gln Lys Gln Leu Gln Ser Leu Ile
225 230 235 240

Pro Phe Val Gln Leu Pro Pro Ser Asp Ser Val Cys Glu Glu Tyr Gly
245 250 255

Leu Ser Cys Ser Asp Ala Leu His Asn Leu Phe Tyr Ile Ser Gly Phe
260 265 270

Ile Ser Gln Cys Thr His Gly Val Gly Arg Ser Ser Thr Asp Arg Gln
275 280 285

Phe Phe Phe Ile Asn Arg Arg Pro Cys Asp Pro Ala Lys Val Cys Arg
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Leu Val Asn Glu Val Tyr His Met Tyr Asn Arg His Gln Tyr Pro Phe
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Val Val Leu Asn Ile Ser Val Asp Ser Glu Cys Val Asp Ile Asn Val
325 330 335

Thr Pro Asp Lys Arg Gln Ile Leu Leu Gln Glu Glu Lys Leu Leu Leu
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Ala Val Leu Lys Thr Ser Leu Ile Gly Met Phe Asp Ser Asp Val Asn
355 360 365

Lys Leu Asn Val Ser Gln Gln Pro Leu Leu Asp Val Glu Gly Asn Leu
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Ile Lys Met His Ala Ala Asp Leu Glu Lys Pro Met Val Glu Lys Gln
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Asp Gln Ser Pro Ser Leu Arg Thr Gly Glu Glu Lys Lys Asp Val Ser
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Ile Ser Arg Leu Arg Glu Ala Phe Ser Leu Arg His Thr Thr Glu Asn

420 425 430

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Gln Lys Arg Gly Met Leu Ser Ser Ser Thr Ser Gly Ala Ile Ser Asp
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Lys Gly Val Leu Arg Pro Gln Lys Glu Ala Val Ser Ser Ser His Gly
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Gly Ser Thr Ser Val Asp Ser Glu Gly Phe Ser Ile Pro Asp Thr Gly
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Lys Arg Phe Lys Lys Glu Glu Ile Leu Ser Ser Ser Asp Ile Cys Gln
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Lys Leu Val Asn Thr Gln Asp Met Ser Ala Ser Gln Val Asp Val Ala
595 600 605

Val Lys Ile Asn Lys Lys Val Val Pro Leu Asp Phe Ser Met Ser Ser
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Leu Ala Lys Arg Ile Lys Gln Leu His His Glu Ala Gln Gln Ser Glu
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Gly Glu Gln Asn Tyr Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu
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Asn Gln Ala Ala Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Thr Met
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Phe Ala Glu Met Glu Ile Ile Gly Gln Phe Asn Leu Gly Phe Ile Ile

675 680 685

Thr Lys Leu Asn Glu Asp Ile Phe Ile Val Asp Gln His Ala Thr Asp
690 695 700

Glu Lys Tyr Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Gly
705 710 715 720

Gln Arg Leu Ile Ala Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu
725 730 735

Ala Val Leu Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly Phe Asp
740 745 750

Phe Val Ile Asp Glu Asn Ala Pro Val Thr Glu Arg Ala Lys Leu Ile
755 760 765

Ser Leu Pro Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Val Asp
770 775 780

Glu Leu Ile Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro
785 790 795 800

Ser Arg Val Lys Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val
805 810 815

Met Ile Gly Thr Ala Leu Asn Thr Ser Glu Met Lys Lys Leu Ile Thr
820 825 830

His Met Gly Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro
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Leu Asp Ala Gly Ala Thr Ser Val Asp Val Lys Leu Glu Asn Tyr Gly
         35                  40                  45

Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val
     50                  55                  60

Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
 65                  70                  75                  80

His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
                 85                  90                  95

Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
            100                 105                 110

Ala Ala Asp Asn Phe Ser Thr Gln Tyr Val Leu Asp Gly Ser Gly His
        115                 120                 125

Ile Leu Ser Gln Lys Pro Ser His Leu Gly Gln Gly Thr Thr Val Thr
    130                 135                 140

Ala Leu Arg Leu Phe Lys Asn Leu Pro Val Arg Lys Gln Phe Tyr Ser
145                 150                 155                 160

Thr Ala Lys Lys Cys Lys Asp Glu Ile Lys Lys Ile Gln Asp Leu Leu
                165                 170                 175

Met Ser Phe Gly Ile Leu Lys Pro Asp Leu Arg Ile Val Phe Val His
            180                 185                 190

Asn Lys Ala Val Ile Trp Gln Lys Ser Arg Val Ser Asp His Lys Met
        195                 200                 205

Ala Leu Met Ser Val Leu Gly Thr Ala Val Met Asn Asn Met Glu Ser
    210                 215                 220

Phe Gln Tyr His Ser Glu Glu Ser Gln Ile Tyr Leu Ser Gly Phe Leu
225                 230                 235                 240

Pro Lys Cys Asp Ala Asp His Ser Phe Thr Ser Leu Ser Thr Pro Glu
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Leu Lys Leu Ile Arg His His Tyr Asn Leu Lys Cys Leu Lys Glu Ser
275 280 285

Thr Arg Leu Tyr Pro Val Phe Phe Leu Lys Ile Asp Val Pro Thr Ala
290 295 300

Asp Val Asp Val Asn Leu Thr Pro Asp Lys Ser Gln Val Leu Leu Gln
305 310 315 320

Asn Lys Glu Ser Val Leu Ile Ala Leu Glu Asn Leu Met Thr Thr Cys
325 330 335

Tyr Gly Pro Leu Pro Ser Thr Asn Ser Tyr Glu Asn Asn Lys Thr Asp
340 345 350

Val Ser Ala Ala Asp Ile Val Leu Ser Lys Thr Ala Glu Thr Asp Val
355 360 365

Leu Phe Asn Lys Val Glu Ser Ser Gly Lys Asn Tyr Ser Asn Val Asp
370 375 380

Thr Ser Val Ile Pro Phe Gln Asn Asp Met His Asn Asp Glu Ser Gly
385 390 395 400

Lys Asn Thr Asp Asp Cys Leu Asn His Gln Ile Ser Ile Gly Asp Phe
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Gly Tyr Gly His Cys Ser Ser Glu Ile Ser Asn Ile Asp Lys Asn Thr
420 425 430

Lys Asn Ala Phe Gln Asp Ile Ser Met Ser Asn Val Ser Trp Glu Asn
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Ser Gln Thr Glu Tyr Ser Lys Thr Cys Phe Ile Ser Ser Val Lys His
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Thr Gln Ser Glu Asn Gly Asn Lys Asp His Ile Asp Glu Ser Gly Glu
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Asn Glu Glu Glu Ala Gly Leu Glu Asn Ser Ser Glu Ile Ser Ala Asp
485 490 495

Glu Trp Ser Arg Gly Asn Ile Leu Lys Asn Ser Val Gly Glu Asn Ile
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Glu Pro Val Lys Ile Leu Val Pro Glu Lys Ser Leu Pro Cys Lys Val
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Ser Asn Asn Asn Tyr Pro Ile Pro Glu Gln Met Asn Leu Asn Glu Asp
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Ser Cys Asn Lys Lys Ser Asn Val Ile Asp Asn Lys Ser Gly Lys Val
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565 570 575

Ala Ser Ala Leu Phe Val Gln Asp His Arg Pro Gln Phe Leu Ile Glu
580 585 590

Asn Pro Lys Thr Ser Leu Glu Asp Ala Thr Leu Gln Ile Glu Glu Leu
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Trp Lys Thr Leu Ser Glu Glu Glu Lys Leu Lys Tyr Glu Glu Lys Ala
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Thr Lys Asp Leu Glu Arg Tyr Asn Ser Gln Met Lys Arg Ala Ile Glu
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Gln Glu Ser Gln Met Ser Leu Lys Asp Gly Arg Lys Lys Ile Lys Pro
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Thr Ser Ala Trp Asn Leu Ala Gln Lys His Lys Leu Lys Thr Ser Leu
660 665 670

Ser Asn Gln Pro Lys Leu Asp Glu Leu Leu Gln Ser Gln Ile Glu Lys
675 680 685

Arg Arg Ser Gln Asn Ile Lys Met Val Gln Ile Pro Phe Ser Met Lys
690 695 700

Asn Leu Lys Ile Asn Phe Lys Lys Gln Asn Lys Val Asp Leu Glu Glu
705 710 715 720

Lys Asp Glu Pro Cys Leu Ile His Asn Leu Arg Phe Pro Asp Ala Trp
725 730 735

Leu Met Thr Ser Lys Thr Glu Val Met Leu Leu Asn Pro Tyr Arg Val
740 745 750

Glu Glu Ala Leu Leu Phe Lys Arg Leu Leu Glu Asn His Lys Leu Pro
755 760 765

Ala Glu Pro Leu Glu Lys Pro Ile Met Leu Thr Glu Ser Leu Phe Asn
770 775 780

Gly Ser His Tyr Leu Asp Val Leu Tyr Lys Met Thr Ala Asp Asp Gln
785 790 795 800

Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp Pro Arg Leu Thr Ala Asn
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Gly Phe Lys Ile Lys Leu Ile Pro Gly Val Ser Ile Thr Glu Asn Tyr
820 825 830

Leu Glu Ile Glu Gly Met Ala Asn Cys Leu Pro Phe Tyr Gly Val Ala
835 840 845

Asp Leu Lys Glu Ile Leu Asn Ala Ile Leu Asn Arg Asn Ala Lys Glu
850 855 860

Val Tyr Glu Cys Arg Pro Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu
865 870 875 880

Ala Val Arg Leu Ser Arg Gln Leu Pro Met Tyr Leu Ser Lys Glu Asp
885 890 895

Ile Gln Asp Ile Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile
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Lys Glu Cys Val His Gly Arg Pro Phe Phe His His Leu Thr Tyr Leu
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Pro Glu Thr Thr
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Asp Ala Leu Leu Ala Ala Arg Glu Val Phe Lys Thr Gln Gly Val Ile
50 55 60

Lys Tyr Met Gly Pro Ala Gly Ala Lys Asn Leu Gln Ser Val Val Leu
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Ser Lys Met Asn Phe Glu Ser Phe Val Lys Asp Leu Leu Leu Val Arg
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Gln Tyr Arg Val Glu Val Tyr Lys Asn Arg Ala Gly Asn Lys Ala Ser
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Lys Glu Asn Asp Trp Tyr Leu Ala Tyr Lys Ala Ser Pro Gly Asn Leu
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Ser Gln Phe Glu Asp Ile Leu Phe Gly Asn Asn Asp Met Ser Ala Ser
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Ile Gly Val Val Gly Val Lys Met Ser Ala Val Asp Gly Gln Arg Gln
145 150 155 160

Val Gly Val Gly Tyr Val Asp Ser Ile Gln Arg Lys Leu Gly Leu Cys
165 170 175

Glu Phe Pro Asp Asn Asp Gln Phe Ser Asn Leu Glu Ala Leu Leu Ile
180 185 190

Gln Ile Gly Pro Lys Glu Cys Val Leu Pro Gly Gly Glu Thr Ala Gly
195 200 205

Asp Met Gly Lys Leu Arg Gln Ile Ile Gln Arg Gly Gly Ile Leu Ile
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Thr Glu Arg Lys Lys Ala Asp Phe Ser Thr Lys Asp Ile Tyr Gln Asp

225 230 235 240

Leu Asn Arg Leu Leu Lys Gly Lys Lys Gly Glu Gln Met Asn Ser Ala
245 250 255

Val Leu Pro Glu Met Glu Asn Gln Val Ala Val Ser Ser Leu Ser Ala
260 265 270

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275 280 285

Phe Glu Leu Thr Thr Phe Asp Phe Ser Gln Tyr Met Lys Leu Asp Ile
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Thr Gly Ser Gln Ser Leu Ala Ala Leu Leu Asn Lys Cys Lys Thr Pro
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340 345 350

Lys Asn Arg Ile Glu Glu Arg Leu Asn Leu Val Glu Ala Phe Val Glu
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370 375 380

Pro Asp Leu Asn Arg Leu Ala Lys Lys Phe Gln Arg Gln Ala Ala Asn
385 390 395 400

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Arg Ser Ala Thr Lys Asp Ser Leu Ile Ile Ile Asp Glu Leu Gly Arg

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Ile Met Glu Pro Ala Ala Lys Lys Cys Tyr Leu Glu Arg Glu Gln Gly
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35 40 45

Val Ile Val Lys Glu Gly Gly Leu Lys Leu Ile Gln Ile Gln Asp Asn
50 55 60

Gly Thr Gly Ile Arg Lys Glu Asp Leu Asp Ile Val Cys Glu Arg Phe
65 70 75 80

Thr Thr Ser Lys Leu Gln Ser Phe Glu Asp Leu Ala Ser Ile Ser Thr
85 90 95

Tyr Gly Phe Arg Gly Glu Ala Leu Ala Ser Ile Ser His Val Ala His
100 105 110

Val Thr Ile Thr Thr Lys Thr Ala Asp Gly Lys Cys Ala Tyr Arg Ala
115 120 125

Ser Tyr Ser Asp Gly Lys Leu Lys Ala Pro Pro Lys Pro Cys Ala Gly
130 135 140

Asn Gln Gly Thr Gln Ile Thr Val Glu Asp Leu Phe Tyr Asn Ile Ala
145 150 155 160

Thr Arg Arg Lys Ala Leu Lys Asn Pro Ser Glu Glu Tyr Gly Lys Ile
165 170 175

Leu Glu Val Val Gly Arg Tyr Ser Val His Asn Ala Gly Ile Ser Phe
180 185 190

Ser Val Lys Lys Gln Gly Glu Thr Val Ala Asp Val Arg Thr Leu Pro
195 200 205

Asn Ala Ser Thr Val Asp Asn Ile Arg Ser Ile Phe Gly Asn Ala Val
210 215 220

Ser Arg Glu Leu Ile Glu Ile Gly Cys Glu Asp Lys Thr Leu Ala Phe
225 230 235 240

Lys Met Asn Gly Tyr Ile Ser Asn Ala Asn Tyr Ser Val Lys Lys Cys
245 250 255

Ile Phe Leu Leu Phe Ile Asn His Arg Leu Val Glu Ser Thr Ser Leu
260 265 270

Arg Lys Ala Ile Glu Thr Val Tyr Ala Ala Tyr Leu Pro Lys Asn Thr
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His Pro Phe Leu Tyr Leu Ser Leu Glu Ile Ser Pro Gln Asn Val Asp
290 295 300

Val Asn Val His Pro Thr Lys His Glu Val His Phe Leu His Glu Glu
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325 330 335

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Ser Ser Thr Ser Gly Ser Ser Asp Lys Val Tyr Ala His Gln Met Val
370 375 380

Arg Thr Asp Ser Arg Glu Gln Lys Leu Asp Ala Phe Leu Gln Pro Leu
385 390 395 400

Ser Lys Pro Leu Ser Ser Gln Pro Gln Ala Ile Val Thr Glu Asp Lys
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Thr Asp Ile Ser Ser Gly Arg Ala Arg Gln Gln Asp Glu Glu Met Leu
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Glu Leu Pro Ala Pro Ala Glu Val Ala Ala Lys Asn Gln Ser Leu Glu
435 440 445

Gly Asp Thr Thr Lys Gly Thr Ser Glu Met Ser Glu Lys Arg Gly Pro
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Ile Asn Glu Gln Gly His Glu Val Leu Arg Glu Met Leu His Asn His
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Ser Phe Val Gly Cys Val Asn Pro Gln Trp Ala Leu Ala Gln His Gln
    530                 535                 540

Thr Lys Leu Tyr Leu Leu Asn Thr Thr Lys Leu Ser Glu Glu Leu Phe
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Tyr Gln Ile Leu Ile Tyr Asp Phe Ala Asn Phe Gly Val Leu Arg Leu
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Ser Glu Pro Ala Pro Leu Phe Asp Leu Ala Met Leu Ala Leu Asp Ser
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Pro Glu Ser Gly Trp Thr Glu Glu Asp Gly Pro Lys Glu Gly Leu Ala
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Glu Tyr Ile Val Glu Phe Leu Lys Lys Lys Ala Glu Met Leu Ala Asp
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Tyr Phe Ser Leu Glu Ile Asp Glu Glu Gly Asn Leu Ile Gly Leu Pro
625                 630                 635                 640

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Ile Leu Arg Leu Ala Thr Glu Val Asn Trp Asp Glu Glu Lys Glu Cys
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Phe Glu Ser Leu Ser Lys Glu Cys Ala Met Phe Tyr Ser Ile Arg Lys
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Gln Tyr Ile Ser Glu Glu Ser Thr Leu Ser Gly Gln Gln Ser Glu Val
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Pro Gly Ser Ile Pro Asn Ser Trp Lys Trp Thr Val Glu His Ile Val
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Phe Glu Arg Cys
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<212> PRT
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        35                  40                  45

Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp
    50                  55                  60

Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
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Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
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Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser
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<211> 426
<212> DNA
<213> Homo sapiens

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acttga 426

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30588

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :424/93.2, 130.1; 435 /69.1, 410, 440; 536/24.5; 800/25
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2, 130.1; 435 /69.1, 410, 440; 536/24.5; 800/25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS, MEDLINE, BIOSIS, EMBASE, CAPLUS, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KONG, Q. et al. PMS2-deficiency diminishes hypermutation of a lambda1 transgene in young but not older mice. Molecular Immunology. 1999, Vol. 36, pages 83-91, entire document.	1-4, 12-15, 22-25, 30-56
Y	VORA K.A. et al. Severe Attenuation of the B Cell Immune Response In Msh2-deficient Mice. Journal of Experimental Medicine. February 1999, Vol. 189, No. 3, pages 471-481, entire document.	1-2, 7-8, 12-13, 22-23, 30-56.
Y	WINTER, D.B. ET AL. Altered spectra of hypermutation in antibodies from mice deficient for the DNA mismatch repair protein PMS2. Proc. Natl. Acad. Sci., USA. June 1998, Vol. 95, pages 6953-6958, entire document.	1-4, 12-15, 22-25, 30-56

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search 05 JANUARY 2001	Date of mailing of the international search report 12 MAR 2001
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30588

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHRADER, C.E. et al. Reduced Isotype Switching in Splenic B Cells From Mice Deficient in Mismatch Repair Enzymes. Journal of Experimental Medicine. 1999, Vol. 190, No. 3, pages 323-330, entire document.	1-4, 6, 12-13, 17 22-23,27, 30-56

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30588

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A01N 63/00; A61K 39/395, 48/00; C07H 21/04; C12N 5/00, 15/00; C12P 21/06